

Molecular Pathology Selected Abstracts, 12/16

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ANXA1 as a predictive biomarker for resistance to trastuzumab in breast cancer

Treatment with the HER2-targeting antibody trastuzumab (Herceptin) is a key component of therapy for women with HER2-positive breast cancer. However, a subset of women with advanced disease shows initial or acquired resistance to therapy, although the mechanisms that control this resistance are largely unknown. Some studies have suggested that activation of the PI3K/mTOR signaling pathway may be responsible for trastuzumab resistance. The authors of this study undertook a multistep molecular approach to identify the pathways involved in HER2-therapy resistance, with an emphasis on breast cancer as a model system. They first performed five independent genome-wide shRNA screens using barcode methodology in HER2-amplified breast cancer cell lines to determine which genes, when reduced or lost, could cause resistance to HER2-targeted therapy. This analysis indicated that loss of *ARID1A*, a subunit of the SWI/SNF chromatin-remodeling complex and a gene that is frequently mutated in cancer, could induce resistance to trastuzumab and to the mTOR inhibitor AZD8055. Loss of *ARID1A* increased phosphorylation of AKT, an indicator of PI3K/mTOR pathway activation. To identify which protein or pathway functioned downstream of *ARID1A*, the authors next performed transcriptome sequencing analysis (RNA-seq) in cells in which *ARID1A* was depleted to identify proteins inversely associated with *ARID1A* expression. *ANXA1*, a calcium/phospholipid binding protein that has been implicated in cancer progression, appeared to be a promising downstream regulator in cell lines and screens based on data from The Cancer Genome Atlas database. In addition, chromatin immunoprecipitation sequencing experiments showed that *ARID1A* appeared to regulate binding of BRG1 to the promoter of *ANXA1*, and high *ANXA1* levels correlated with increases in phosphorylation of AKT and resistance to HER2-targeted therapy. Therefore, it appears that *ARID1A* loss is associated with increased *ANXA1* expression, phosphorylation of AKT, activation of the PI3K/mTOR pathway, and resistance to trastuzumab. Inhibition of AKT by MK2206 could reverse resistance to HER2/PI3K/mTOR-targeted therapy in these cell lines, suggesting that *ARID1A* and *ANXA1* expression could be used to determine whether combination therapy with AKT inhibitors could be considered in patients who develop resistance to trastuzumab. The authors translated their findings to two clinical studies in which breast cancer patients were treated with chemotherapy with or without trastuzumab—the FinHER phase III clinical trial and the Responsify dataset. In both studies, high *ANXA1* gene-expression levels were associated with limited response or no response to HER2-targeted therapy, and *ANXA1* had no separate effect on patient outcomes. These results suggest that *ANXA1* may be a useful predictive biomarker for determining response to HER2-targeted therapy. If validated in future studies, incorporation of this biomarker may add significant value to the current panels performed in the pathology laboratory for breast cancer patients and to personalized cancer care.

Berns K, Sonnenblick A, Gennissen A, et al. Loss of *ARID1A* activates *ANXA1*, which serves as a predictive biomarker for trastuzumab resistance. *Clin Care Res*. 2016;22:5238–5248.

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Development of a child-parent screening paradigm for familial hypercholesterolemia

Familial, or hereditary, hypercholesterolemia is a significant risk factor for early onset cardiovascular disease that includes a 100-fold increase in the risk of coronary heart disease in patients younger than 40 years of age. Patients with familial hypercholesterolemia have persistent and markedly elevated cholesterol levels that are inherited and, therefore, multigenerational. In a subset of patients, this condition is likely influenced by a mutation in several known genes related to cholesterol metabolism, including the *LDLR*, *APOB*, and *PCSK9* genes, although there appear to be other contributory genetic alterations that have not yet been identified. However, not all patients with a mutation in one of these genes develop hypercholesterolemia, leading to challenges in defining the condition and screening for it. The authors conducted a study in the United Kingdom to assess the efficacy and feasibility of screening for familial hypercholesterolemia in a primary care practice. During routine immunization visits, they screened more than 10,000 one- and two-year-old children for total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides. Each child was also tested for the 48 most common mutations in familial hypercholesterolemia (FH48) using single-nucleotide polymorphism (SNP) genotyping. A child was considered to have a positive screening test if markedly elevated cholesterol levels were detected at two visits months apart or if a markedly elevated cholesterol level was associated with a mutation. Sanger sequencing and multiplex ligation-dependent probe amplification were performed for the *LDLR* gene, exon 26 of the *APOB* gene, and exon 7 of the *PCSK9* gene in the subset of patients who had elevated cholesterol but were mutation-negative on FH48 testing to expand the screen for gene mutations. The results from this screening approach identified 40 children with a positive result for familial hypercholesterolemia, including 32 children with a mutation and eight children with no known mutation. The parents of these 40 children were tested to identify shared mutations or abnormally elevated cholesterol levels, or both. None of the parents found to have familial hypercholesterolemia were being actively monitored for the condition at the time of screening. However, once identified, 90 percent of them began statin therapy as a preventative measure to lower cholesterol levels. Based on the results of this analysis, several potential benefits may be derived using the paradigm for population-based screening of child and parent for familial hypercholesterolemia during routine office visits. First, the screening has the potential to identify family members with this condition who possibly would have otherwise suffered adverse medical consequences, including early cardiac disease, and to spur intervention in the parent immediately and the child once the latter reaches adolescence. Second, screening during routine office visits eliminates the costs and time associated with additional clinic visits. Third, this approach incorporates the persistent marked elevation of cholesterol as an indicator of familial hypercholesterolemia, which has the potential to identify genetic alterations that have not yet been described in this patient population. The authors concluded that additional studies to determine how best to apply this approach in the United States could be valuable to prevent early death from a relatively common condition in parents and children.

Wald DS, Bestwick JP, Morris JK, et al. Child-parent familial hypercholesterolemia screening in primary care. *N Engl J Med*. 2016;375:1628-1637.

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